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(54) NOVEL MAGNETIC RESONANCE IMAGING AGENTS

KERNRESONANZABBILDENDE MITTEL

AGENTS D'IMAGERIE A RESONANCE MAGNETIQUE

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Description

This invention relates to nuclear magnetic resonance (NMR) imaging and, more particularly, to methods and compositions for enhancing NMR imaging.

The recently developed technique of NMR imaging encompasses the detection of certain atomic nuclei utilizing magnetic fields and radio-frequency radiation. It is similar in some respects to x-ray computed tomography (CT) in providing a cross-sectional display of the body organ anatomy with excellent resolution of soft tissue detail. As currently used, the images produced constitute a map of the distribution density of protons and/or their relaxation times in organs and tissues. The technique of NMR imaging is advantageously non-invasive as it avoids the use of ionizing radiation.

While the phenomenon of NMR was discovered in 1945, it is only relatively recently that it has found application as a means of mapping the internal structure of the body as a result of the original suggestion of Lauterbur (Nature, 242, 190-191 (1973)). The fundamental lack of any known hazard associated with the level of the magnetic and radio-frequency fields that are employed renders it possible to make repeated scans on vulnerable individuals. Additionally, any scan plane can readily be selected, including transverse, coronal and sagittal sections.

In an NMR experiment, the nuclei under study in a sample (e.g. protons) are irradiated with the appropriate radio-frequency (RF) energy in a highly uniform magnetic field. These nuclei, as they relax, subsequently emit RF at a sharp resonance frequency. The resonance frequency of the nuclei depends on the applied magnetic field.

According to known principles, nuclei with appropriate spin, when placed in an applied magnetic field (B, expressed generally in units of gauss or Tesla (10^4 gauss)) align in the direction of the field. In the case of protons, these nuclei precess at a frequency, f, of 42.6 MHz at a field strength of 1 Tesla. At this frequency, an RF pulse of radiation will excite the nuclei and can be considered to tip the net magnetization out of the field direction, the extent of this rotation being determined by the pulse duration and energy. After the RF pulse, the nuclei "relax" or return to equilibrium with the magnetic field, emitting radiation at the resonant frequency. The decay of the emitted radiation is characterized by two relaxation times, i.e., T_1, the spin-lattice relaxation time or longitudinal relaxation time, that is, the time taken by the nuclei to return to equilibrium along the direction of the externally applied magnetic field, and T_2, the spin-spin relaxation time associated with the dephasing of the initially coherent precession of individual proton spins. These relaxation times have been established for various fluids, organs and tissues in different species of mammals.

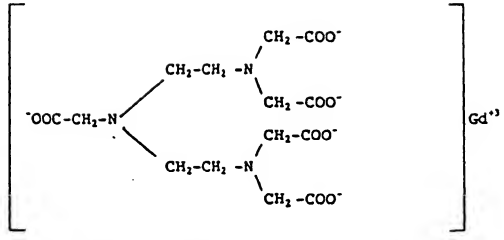
In NMR imaging, scanning planes and slice thicknesses can be selected. This selection permits high quality transverse, coronal and sagittal images to be obtained directly. The absence of any moving parts in NMR imaging equipment promotes a high reliability. It is believed that NMR imaging has a greater potential than CT for the selective examination of tissue characteristics in view of the fact that in CT, x-ray attenuation coefficients alone determine image contrast, whereas at least four separate variables (T_1, T_2, proton density and flow) may contribute to the NMR signal. For example, it has been shown (Damadian, Science, 171, 1151 (1971)) that the values of the T_1 and T_2 relaxation in tissues are generally longer by about a factor of 2 in excised specimens of neoplastic tissue compared with the host tissue.

By reason of its sensitivity to subtle physicochemical differences between organs and/or tissues, it is believed that NMR may be capable of differentiating different tissue types and in detecting diseases which induce physicochemical changes that may not be detected by x-ray or CT which are only sensitive to differences in the electron density of tissue.

As noted above, two of the principal imaging parameters are the relaxation times, T_1 and T_2. For protons (or other appropriate nuclei), these relaxation times are influenced by the environment of the nuclei (e.g., viscosity, temperature, and the like). These two relaxation phenomena are essentially mechanisms whereby the initially imparted radiofrequency energy is dissipated to the surrounding environment. The rate of this energy loss or relaxation can be influenced by certain other nuclei which are paramagnetic. Chemical compounds incorporating these paramagnetic nuclei may substantially alter the T_1 and T_2 values for nearby protons. The extent of the paramagnetic effect of a given chemical compound is a function of the environment within which it finds itself.

In general, paramagnetic divalent or trivalent ions of elements with an atomic number of 21 to 29, 42 to 44 and 58 to 70 have been found effective as NMR image contrasting agents. Suitable such ions include chromium (III), manganese (II), manganese (III), iron (III), iron (II), cobalt (II), nickel (II), copper (II), praseodymium (III), neodymium (III), samarium (III) and ytterbium (III). Because of their very strong magnetic moments, gadolinium (III), terbium (III), dysprosium (III), holmium (III) and erbium (III) are preferred. Gadolinium (III) ions have been particularly preferred as NMR image contrasting agents.

Typically, the divalent and trivalent paramagnetic ions have been administered in the form of complexes with organic complexing agents. Such complexes provide the paramagnetic ions in a soluble, non-toxic form, and facilitate their rapid clearance from the body following the imaging procedure. Gries et al., US-A-4,647,447, disclose complexes of various paramagnetic ions with conventional aminocarboxylic acid complexing agents. A preferred complex disclosed by Gries et al. is the complex of gadolinium (III) with diethylenetriaminepentaacetic acid ("DTPA"). This complex may be represented by the formula:



Paramagnetic ions, such as gadolinium (III), have been found to form strong complexes with DTPA. These complexes do not dissociate substantially in physiological aqueous fluids. The complexes have a net charge of -2, and generally are administered as soluble salts. Typical such salts are the sodium and N-methylglucamine salts.

The administration of ionizable salts is attended by certain disadvantages. These salts can raise the in vivo ion concentration and cause localized disturbances in osmolality, which in turn, can lead to edema and other undesirable reactions.

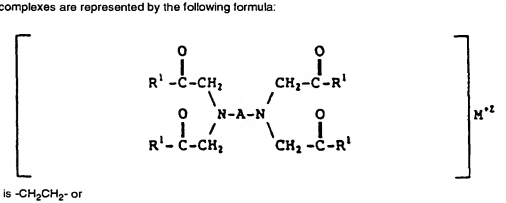
Efforts have been made to design non-ionic paramagnetic ion complexes. In general, this goal has been achieved by converting one or more of the free carboxylic acid groups of the complexing agent to neutral, non-ionizable groups. For example, S.C. Quay, in US-A-4,687,658 and 4,687,659, discloses alkylester and alkylamide derivatives, respectively, of DTPA complexes. Similarly, DE-A-3324235 and 3324236 disclose mono- and polyhydroxyalkylamide derivatives of DTPA and their use as complexing agents for paramagnetic ions.

The nature of the derivative used to convert carboxylic acid groups to non-ionic groups can have a significant impact on solubility. For example, derivatizing the carboxylic acid groups with hydrophobic alkylamide groups substantially decreases the water solubility of the complex. The solubility of the complexes in physiological fluids can, in turn, affect the tissue selectivity of the complex. Hydrophilic complexes tend to concentrate in the interstitial fluids, whereas hydrophobic complexes tend to associate with cells. Thus, differences in hydrophilicity can lead to different applications of the compounds. See, for example, Weinmann et al., AJR, 142, 679 (Mar. 1984) and Brasch et al., AJR, 142, 625 (Mar. 1984).

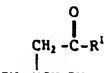
Thus, a need continues to exist for new and structurally diverse non-ionic complexes of paramagnetic ions for use as NMR imaging agents.

Summary of the Invention

The present invention provides novel complexing agents and complexes of complexing agents with paramagnetic ions. The complexes are represented by the following formula:



wherein A is $-CH_2CH_2-$ or



and M^{+Z} is a paramagnetic ion of an element with an atomic number of 21-29, 42-44 or 58-70, and a valence, Z, of +2 or +3; the R^1 groups may be the same or different and are selected from the group consisting of $-O^-$ and

wherein R^2 is alkoxyalkyl in which the alkoxy portion contains 1 or 2 carbon atoms and the alkyl portion contains from 2 to 5 carbon atoms, or is $-(CH_2CH_2O)_n-R^4$ wherein n is 1-10 and R^4 is H (except when n = 1), alkyl having 1 to 8 carbon atoms, or an aryl group which is unsubstituted or substituted with hydroxy and R^3 is H, R^2, alkyl having from 1 to 8 carbon atoms, hydroxy, alkoxy having from 1-8 carbon atoms, cycloalkyl with up to 10 carbon atoms, or an aryl group which is unsubstituted or substituted with hydroxy, carboxyl, halogen, alkoxy having from 1 to 8 carbon atoms or alkyl having from 1 to 8 carbon atoms, wherein 2 or 3 of the R^1 groups are $-O^-$ and the remainder of the R^1 groups are

groups. The formula of the complexing agents is set forth in Claim 1.

Also disclosed is a method of performing an NMR diagnostic procedure which involves administering to a warm-blooded animal an effective amount of the above-described complex and then exposing the warm-blooded animal to an NMR imaging procedure, thereby imaging at least a portion of the body of the warm-blooded animal.

Detailed Description of the Invention

The complexing agents employed in this invention are derivatives of the well-known chelating agents, DTPA and ethylenediaminetetraacetic acid ("EDTA"). In these derivatives, free carboxylic acid groups of DTPA (those not involved in the formation of coordination bonds with the paramagnetic ion) are converted to amide groups. Thus, if the paramagnetic ion is trivalent, two of the carboxylic acid groups of DTPA or one of the carboxylic acid groups of EDTA will be derivatized to the amide form. Likewise, if the paramagnetic ion is divalent, three of the carboxylic acid groups of DTPA or two of the carboxylic acid groups of EDTA will be derivatized to the amide form. When reacted with a divalent or trivalent paramagnetic ion, the resulting complexes are substantially non-ionic and neutral.

The amide derivatives of DTPA and EDTA are prep;,red in a conventional manner. In general, they are prepared by reacting a stoichiometric amount of an amine having the general formula

wherein R^2 and R^3 are as defined above, with a reactive derivative of DTPA or EDTA under amide-forming conditions. Such reactive derivatives include, for example, anhydrides, mixed anhydrides and acid chlorides. In one embodiment, the reactions are conducted in an organic solvent at an elevated temperature. Suitable solvents include those in which the reactants are sufficiently soluble and which are substantially unreactive with the reactants and products. Lower aliphatic alcohols, ketones, ethers, esters, chlorinated hydrocarbons, benzene, toluene, xylene, lower aliphatic hydro-

carbons, and the like may advantageously be used as reaction solvents. Examples of such solvents are methanol, ethanol, propanol, butanol, pentanol, acetone, methylethyl ketone, diethylketone, methyl acetate, ethyl acetate, chloroform, methylene chloride, dichloroethane, hexane, heptane, octane, decane, and the like. If a DTPA or EDTA acid chloride is used as the starting material, then the reaction solvent advantageously is one which does not contain reactive functional groups, such as hydroxyl groups, as these solvents can react with the acid chlorides, thus producing unwanted by-products.

The reaction temperature may vary widely, depending upon the starting materials employed, the nature of the reaction solvent and other reaction conditions. Such reaction temperatures may range, for example, from about 0° C to about 150° C, preferably from about 30° C to about 70° C.

Following reaction of the reactive DTPA or EDTA derivative with the amine, any remaining anhydride or acid chloride groups can be hydrolyzed to the carboxylate groups by adding a stoichiometric excess of water to the reaction mixture and heating for a short time.

The alkoxyalkylamine advantageously contains from about 2 to about 6 carbon atoms. In preferred amines, the alkoxy portion contains about 1-2 carbon atoms and the alkyl portion contains from about 2 to about 5 carbon atoms. Such amines include, for example, methoxyethylamine, methoxypropylamine, methoxybutylamine, methoxypentylamine, ethoxyethylamine, ethoxypropylamine, ethoxybutylamine, and mixtures thereof. A particularly preferred amine is methoxyethylamine.

Preferred secondary amine compounds for reaction include amines with repeating alkoxy units such as $-(CH_2CH_2O)$. In the formula given above, preferred compounds are produced when R^2 is $-(CH_2CH_2O)_n-R^4$, R^4 is as defined above and R^3 is an aryl group optionally substituted with hydroxy, carboxyl, alkoxy having 1 to 8 carbons, alkyl having 1 to 8 carbons or halogen. Preferably, n = 1, 2 or 3 and R^4 is H (except when n = 1) or an alkyl group having from 1 to 5 carbon atoms. Also preferably, R^3 is R^2, H, hydroxy, or an alkyl or alkoxy group having from 1 to about 8 carbon atoms.

The resulting DTPA or EDTA alkoxyalkylamide is recovered from the reaction mixture by conventional procedures. For example, the product may be precipitated by adding a precipitating solvent to the reaction mixture, and recovered by filtration or centrifugation.

The paramagnetic ion is combined with the DTPA di-or trialkoxyalkylamide or EDTA mono- or dialkoxyalkylamide under complex-forming conditions. In general, any of the paramagnetic ions referred to above can be employed in making the complexes of this invention. The complexes can conveniently be prepared by mixing a suitable oxide or salt of the paramagnetic ion with the complexing agent in aqueous solution. To assure complete complex formation, a slight stoichiometric excess of the complexing agent may be used. In addition, an elevated temperature, e.g., ranging from about 20° C to about 100° C, preferably from about 40° C to about 80° C, may be employed to insure complete complex formation. Generally, complete complex formation will occur within a period from a few minutes to a few hours after mixing. The complex may be recovered by precipitation using a precipitating solvent such as acetone, and further purified by crystallization, if desired.

The novel complexes of this invention can be formulated into diagnostic compositions for enteral or parenteral administration. These compositions contain an effective amount of the paramagnetic ion complex along with conventional pharmaceutical carriers and excipients appropriate for the type of administration contemplated. For example, parenteral formulations advantageously contain a sterile aqueous solution or suspension of from about 0.05 to 1.0M of a paramagnetic ion complex according to this invention. Preferred parenteral formulations have a concentration of paramagnetic ion complex of 0.1M to 0.5M. Such solutions also may contain pharmaceutically acceptable buffers and, optionally, electrolytes such as sodium chloride. Advantageously, the compositions may further contain physiologically acceptable non-toxic cations in the form of a gluconate, chloride or other suitable organic or inorganic salts, including suitable soluble complexes with a chelant/ligand to enhance safety. The chelant/ligand desirably is derived from DTPA or EDTA. Such ligands include the ligands set forth above used to complex the paramagnetic and/or heavy metals to provide the complex formulations of this invention. Advantageously, the cation-ligand complex is provided in amounts ranging from about 0.1 mole % to about 15 mole % of the ligand-metal complex. Such physiologically acceptable, non-toxic cations include calcium ions, magnesium ions, zinc ions and the like including mixtures thereof. Calcium ions are preferred. A typical single dosage formulation for parenteral administration has the following composition:

Gadolinium DTPA-di(methoxyethylamide)	330mg/ml
Calcium DTPA-di(methoxyethylamide)	14mg/ml
Distilled Water	q.s. to 1 ml
pH	7.0

Parenteral compositions may be injected directly or mixed with a large volume parenteral composition for systemic administration.

Formulations for enteral administration may vary widely, as is well-known in the art. In general, such formulations are liquids which include an effective amount of the paramagnetic ion complex in aqueous solution or suspension. Such enteral compositions may optionally include buffers, surfactants, thixotropic agents, and the like. Compositions for oral administration may also contain flavoring agents and other ingredients for enhancing their organoleptic qualities.

The diagnostic compositions are administered in doses effective to achieve the desired enhancement of the NMR image. Such doses may vary widely, depending upon the particular paramagnetic ion complex employed, the organs or tissues which are the subject of the imaging procedure, the NMR imaging equipment being used, etc. In general, parenteral dosages will range from about 0.01 to about 1.0 mMol of paramagnetic ion complex per kg of patient body weight. Preferred parenteral dosages range from about 0.05 to about 0.5 mMol of paramagnetic ion complex per kg of patient body weight. Enteral dosages generally range from about 0.5 to about 100 mMol, preferably from about 1.0 to about 20 mMol of paramagnetic ion complex per kg of patient body weight.

The novel NMR image contrasting agents of this invention possess a unique combination of desirable features. The paramagnetic ion complexes exhibit an unexpectedly high solubility in physiological fluids, notwithstanding their substantially non-ionic character. This high solubility allows the preparation of concentrated solutions, thus minimizing the amount of fluid required to be administered. The non-ionic character of the complexes also reduces the osmolarity of the diagnostic compositions, thus preventing undesired edema and other side effects. As illustrated by the data presented below, the compositions of this invention have very low toxicities, as reflected by their high LD_{50} values.

The diagnostic compositions of this invention are used in the conventional manner. The compositions may be administered to a warm-blooded animal either systemically or locally to the organ or tissue to be imaged, and the animal then subjected to the NMR imaging procedure. The compositions have been found to enhance the magnetic resonance images obtained by these procedures. In addition to their utility in magnetic resonance imaging procedures, the complexing agents of this invention may also be employed for delivery of radiopharmaceuticals or heavy metals for x-ray contrast into the body.

The invention is further illustrated by the following examples, which are not intended to be limiting.

Example I

Preparation of N,N"-Bis[N-(2-methoxyethyl)-carbamoylmethyl]diethylenetriamine-N,N',N"-triacetic acid.

A stirred suspension of DTPA-dianhydride (10.8 g, 0.030 mole) in 100 ml. of isopropanol was treated with 2-methoxyethylamine (5.0 g, 0.067 mole). The entire mixture was heated at 50° C for 4 hours in a water bath. The pale yellow solution was filtered through a medium porosity sintered glass funnel to remove undissolved impurities, and the filtrate was taken to dryness under reduced pressure. The resulting amorphous foam was dried (vacuum desiccator) at ambient temperature for 18 hours. The yield of the bis(2-methoxyethylamide) of DTPA was 14.4 g (93.5%). ^{13}C-NMR (22.49 MHz, D_2O, ref. p-dioxane at δ 67.4): δ 173.5, 172.3, 170.4, 71.0, 58.8, 57.9, 57.5, 55.9, 52.4, 52.1, 39.6. Analysis calculated for $C_{20}H_{37}N_5O_{10}$·0.4H_2O: C, 46.67%; H, 7.25%; N, 13.61%. Found: C, 47.15%; H, 7.42%; N, 13.35%.

Example II

Preparation of (N,N"-Bis[N-(2-methoxyethyl)-carbamoylmethyl]diethylenetriamine-N,N',N"-triaceto} gadolinium (III)

A mixture of gadolinium (III) oxide (3.3 g, 0.0091 mole) and bis(2-methoxyethylamide) of DTPA produced by the procedure described in Example I (10.2 g, 0.020 mole) in H_2O (100 ml.) was heated at 60-65° C for 3 hours in a water bath. The pale yellow homogeneous solution was filtered through a fine porosity sintered glass funnel to remove undissolved impurities and the clear filtrate was poured into acetone (2l). The heterogeneous mixture was stirred for 5 minutes and allowed to stand at ambient temperature for 30 minutes. Aqueous acetone was decanted off and the resulting gummy residue was dissolved with methanol (150 ml.). The solution was concentrated under reduced pressure and the complex was precipitated from the solution by adding it to more acetone (1l). The amorphous precipitate was collected, washed with acetone (2 X 100 ml.) and dried. The yield was 11.2 g (80.7%). The pale cream solid was crystallized from a mixture of methanol and tetrahydrofuran to give a colorless solid. It was 97.4% pure by HPLC. Analysis calculated for $C_{20}H_{34}N_5O_{10}Gd$.1.4 H_2O: C, 34.95%; H, 5.41%; N, 10.19%; Gd, 22.88%. Found: C, 35.20%; H, 5.42%;, N, 10.27%; Gd, 22.52%.

Example III

Preparation of N,N"-Bis[N-(2-ethoxyethyl)carbamoylmethyl]diethylenetriamine-N,N',N"-triacetic acid.

The procedure of Example I is repeated in all essential details, except that ethoxyethylamine (5.97 g, 0.067 mole)

is substituted for methoxyethylamine. The procedure produces the title compound in good yield.

Example IV

Preparation of {N,N"-Bis[N-(2-ethoxyethyl)-carbamoylmethyl]diethylenetriamine-N,N',N"-triaceto} gadolinium (III)

The procedure of Example II is repeated in all essential details, except that the bis(2-ethoxyethylamide) of DTPA produced by the procedure described in Example III is substituted in equimolar amount for the bis(2-methoxyethylamide) of DTPA. The procedure produces the title compound in good yield.

Example V

Preparation of {N,N"-Bis[N-(2-methoxyethyl)-carbamoylmethyl]diethylenetriamine-N,N',N"-triaceto} iron (III)

The procedure of Example II is repeated in all essential details, except that iron (III) acetylacetonate is substituted in equimolar amount for gadolinium (III) oxide. The procedure produces the title compound in good yield.

Example VI

Preparation of {N,N"-Bis[N-(2-methoxyethyl)-carbamoylmethyl]diethylenetriamine-N,N',N"-triaceto} holmium (III)

The procedure of Example II is repeated in all essential details, except that holmium (III) oxide is substituted in equimolar amount for gadolinium (III) oxide. The procedure produces the title compound in good yield.

Example VII

Preparation of N,N',N"-Tris[N-(2-methoxyethyl) carbamoylmethyl]-diethylenetriamine-N,N"-diacetic acid

DTPA (1 mol) is dissolved in acetonitrile by adding triethylamine (5 mol) and heating. The solution is cooled to room temperature. While stirring, isobutylchloroformate (3 mol) is added dropwise to this solution. An excess of 2-methoxyethylamine (7 mol) is added immediately and the reaction mixture is stirred until the reaction is complete. The solution is taken to dryness under reduced pressure. The crude product is purified by chromatography on an anion exchange column.

Example VIII

Preparation of {N,N',N"-Tris[N-(2-methoxyethyl)carbamoylmethyl]-diethylenetriamine-N,N"-diaceto} manganese (II)

An excess of the tris(2-methoxyethylamide) of DTPA produced by the procedure described in Example VII is dissolved in water and $MnCO_3$ is added. The mixture is stirred and heated until the solution becomes homogeneous. The solution is taken to dryness under reduced pressure to give the desired product.

Example IX

Preparation of N,N'-Bis[N-(2-methoxyethyl)carbamoylmethyl] ethylenediamine-N,N'-diacetic acid

2-Methoxyethylamine (3.0 g, 0.02 mol) in 100 ml of methanol was treated with EDTA-dianhydride (5.12 g, 0.02 mol). The reaction mixture was stirred for 5 hours and the solids dissolved. The solution was taken to dryness under reduced pressure. The residue was dried under high vacuum to give 8.5 g of glassy solid. Its ^{13}C-NMR spectrum was consistent with the desired structure.

Example X

Preparation of {N,N'-Bis[N-(2-methoxyethyl) carbamoylmethyl]-ethylenediamine-N,N'-diaceto}manganese(II)

A 15% excess of the bis(2-methoxyethylamide) of EDTA produced by the procedure described in Example IX (1.1 g, 0.0026 mol) was dissolved in water (10 ml) and $MnCO_3$ (0.27 g, 0.0023 mol) was added. Upon warming for 30 minutes, the solution became homogeneous. The solution was taken to dryness under reduced pressure. The resulting

glassy solid was very soluble in water.

Example XI

The acute intravenous toxicity of the compound of Example II was determined as follows: ICR mice, at 1 to 4 per dose level, received single intravenous injections of the test substance via a lateral tail vein at the rate of approximately 1 ml/minute. The test substances were at concentrations chosen to result in dose volumes of 5 to 75 ml/kg body weight. Dosing began at a volume of 10 ml/kg. Dose adjustments up or down were made to closely bracket the estimated LD_{50} with 4 animals per group (2 males and 2 females). Observations of the mice were recorded at times 0, 0.5, 1,2,4 and 24 hours and once daily thereafter for up to 7 days post injection. On the 7th day post injection, the mice were euthanized, weighed and necropsied. Abnormal tissues were noted. At this time a decision was made as to whether any histopathology was to be performed and whether or not the tissues should be retained. Necropsies were also performed on mice expiring after 24 hours post-injection, except for dead mice found on the weekends. The LD_{50} values, along with 95% CI were calculated using a modified Behrens-Reed-Meunch method. The results for the complex of Example II are reported below:

LD_{50}:	22.5 mmol/kg 95% Confidence Limits: 17.4 - 29.0 mmol/kg
Sex and Weight Range of Mice:	Males(18.0-20.3 g)
	Females (19.0-21.7 g)

The details of the test results are shown in Table I below. The data demonstrate that the complex of Example II was characterized by a low initial I.v. toxicity (LD_{50} - 27mmol/kg) within the first 24 hours post injection. Two delayed deaths at 27.2 mmol/kg resulted in lowering the LD_{50} to 22.5 mmol/kg. Surviving mice, in general, failed to gain weight during the 7-day post-injection period. Only one gross organ abnormality was noted at necropsy: a "pale" colored liver in a female dosed with 20.4 mmol/kg. No other mice at 20.4 mmol/kg or lower showed similar abnormalities. Thus, these preliminary tests suggest that the formulation has a low order of i.v. toxicity.

Table I

Dose (mmol/kg)	Conc (M)	Deaths: Immediate (0-1 hr)	Deaths: (1-24 hr)	Deaths: Delayed (1-7 days)	Total	Body Weight Change (g)
6.8	0.68	0	0	0	0/2	M:-1.1/F:+2.1
13.6	0.68	0	0	0	0/2	M:+1.6/F:+1.4
20.4	0.68	1	0	0	1/4	M:-1.1/F:-3.2
27.2	0.68	2	0	2	4/4	- -
34.0	0.68	4	-	-	4/4	- -

Example XII

T_1 and T_2 relaxivity curves of the complex of Example II were obtained using a RADX (10 megahertz) NMR analyzer. The RADX analyzer was thermally stabilized at 37° C before performing any T_1 or T_2 measurements. Overall range tuning and mid-range calibration were performed on a 37° C warmed T_1 standard at the beginning of the experiment, according to manufacturer's instructions. Subsequent to calibration, T_1 standards were tested to verify calibration and linearity.

Ten millimolar solutions of the complex were prepared in sterile water for injection ("SWFI") and in 4% human serum albumin ("HSA")/0.9% NaCl. A series of lower concentrations (0.25, 0.50, 1.0, 2.5 and 5.0 mM) were prepared

to form a concentration curve. A sample of each prepared concentration was warmed to 37° C in an NMR sample tube prior to assay. Triplicate T_1 and T_2 values were obtained on each dilution.

Separate linear regressions were determined using the reciprocal T_1 and T_2 mean values for the complex diluted in SWFI and 4% HSA. The relaxivity curves were generated by plotting the reciprocal T_1 or T_2 value against concentration. The following relaxation rates were determined for the complex of Example II:

Relaxation Rate ($mM^{-1}sec^{-1}$)			
T_1		T_2	
H_2O	HSA	H_2O	HSA
4.69	4.40	4.81	6.38

Example XIII

Preparation of N,N"-Bis[N-(2-methoxyethyl)methoxycarbaaoylmethyl]diethylenetriamine-N,N',N"-triacetic acid.

The procedure of Example I is repeated in all essential details, except that N-methoxy-2-methoxyethylamine (7.04 g, 0.067 mole) is substituted for methoxyethylamine. The procedure produces the title compound in good yield.

Example XIV

Preparation of {N,N"-Bis[N-(2-methoxyethyl)methoxycarbamoylmethyl]diethylenetriamine-N,N',N"-triaceto} gadolinium(III)

The procedure of Example II is repeated in all essential details, except that the bis(n-methoxy-2-methoxyethylamide) of DTPA produced by the procedure described in Example XIII is substituted in equimolar amount for the bis (2-methoxyethylamide) of DTPA. The procedure produces the title compound in good yield.

Example XV

Preparation of N,N"-Bis(N,N-di-2-methoxyethylcarbamoylmethyl)diethylenetriamine-N,N',N"-triacetic acid.

The procedure of Example I is repeated in all essential details, except that N,N-di-2-methoxyethylamine (8.91 gr, 0.067 mole) is substituted for methoxyethylamine. The procedure produces the title compound in good yield.

Example XVI

Preparation of [N,N"-Bis(N,N-di-2-methoxyethylcarbamoylmethyl)diethylenetriamine-N,N',N"-triaceto]gadolinium(III)

The procedure of Example II is repeated in all essential details, except that the bis(N,N-di-2-methoxyethylamide) of DTPA produced by the procedure described in Example XV is substituted in equimolar amount for the bis(2-methoxyethylamide) of DTPA. The procedure produces the title compound in good yield.

Example XVII

Preparation of N,N'-Bis[N-2-(2-methoxyethoxy)ethylmethylcarbamoylmethyl]diethylenetriamine-N,N',N"-triacetic acid.

The procedure of Example I is repeated in all essential details, except that N-2-(2-methoxyethoxy)-ethylmethylamine (8.91 g, 0.067 mole) is substituted for methoxyethylamine. The procedure produces the title compound in good yield.

Example XVIII

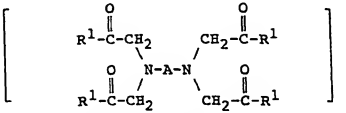
Preparation of {N,N"-Bis[N-2-(2-methoxyethoxy)ethyl methoxycarbamoylmethyl]diethylenetriamine-N,N'N"-triaceto} gadolinium (III).

The procedure of Example II is repeated in all essential details, except that the bis[N-2-(2-methoxyethoxy)ethyl-

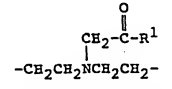
methylamide] of DTPA produced by the procedure described in Example XVII is substituted in equimolar amount for the bis(2-methoxyethylamide) of DTPA. The procedure produces the title compound in good yield.

Claims

1. A complexing agent of the formula:



wherein A is selected from the group consisting of $-CH_2CH_2-$ and



wherein the R^1 groups may be the same or different and are selected from the group consisting of -OH and

wherein R^2 is alkoxyalkyl in which the alkoxy portion contains 1 or 2 carbon atoms and the alkyl portion contains from 2 to 5 carbon atoms, or is $-(CH_2CH_2O)_n-R^4$ wherein n is 1-10 and R^4 is H (except when n = 1), alkyl having 1 to 8 carbon atoms, or aryl unsubstituted or substituted with hydroxy and R^3 is H, R^2, alkyl having from 1 to 8 carbon atoms, hydroxy, alkoxy having 1 to 8 carbon atoms, cycloalkyl with up to 10 carbon atoms, or an aryl group which is optionally substituted with hydroxy, carboxyl, halogen, alkoxy having from 1 to 8 carbon atoms or alkyl having from 1 to 8 carbon atoms, wherein 2 or 3 of the R^1 groups are -OH and the remainder of the R^1 groups are

2. A complexing agent according to claim 1 wherein R^1 is methoxyethylamino, methoxypropylamino, methoxybutylamino, methoxypentylamino, ethoxyethylamino, ethoxypropylamino or ethoxybutylamino.

3. A complexing agent according to claim 1 wherein R^2 is $-(CH_2CH_2O)_n-R^4$, n is 1, 2 or 3 and R^4 is H (except when n = 1) or an alkyl group having 1 to 5 carbon atoms.

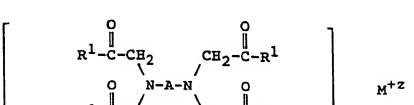
4. A complexing agent according to any one of claims 1 to 3 wherein R^3 is H.

5. A complexing agent according to any one of claims 1 to 3, wherein R^3 is R^2, alkyl having from 1 to 8 carbon atoms, hydroxy, or an alkoxy group having from 1 to 8 carbon atoms.

6. A complexing agent according to any one of claims 1 to 3, wherein R^3 is an aryl group optionally substituted with

hydroxy, carboxyl, halogen, alkoxy having from 1 to 8 carbon atoms or alkyl having from 1 to 8 carbon atoms.

7. A complex of a complexing agent according to any one of claims 1 to 6 and a metal ion M^{z+} and having the following formula:



wherein A has the meaning ascribed to it in claim 1 and R^1 is

as defined in Claim 1 or is $-O^-$, and M^{+z} is a paramagnetic ion of an element with an atomic number of 21-29, 42-44 or 58-70, and a valence, Z, of +2 or +3.

8. A complex according to claim 7, wherein M^{+z} is chromium (III), manganese (II), manganese (III), iron (III), iron (II), cobalt (II), nickel (II), copper (II), praseodymium (III), neodymium (III), samarium (III), ytterbium (III), gadolinium (III), terbium (III), dysprosium (III), holmium (III) or erbium (III).

9. A complex according to claim 7 or claim 8, wherein R^1 is methoxyethylamino and M^{+z} is gadolinium (III).

10. A diagnostic composition suitable for enteral or parenteral administration to a warm-blooded animal, which comprises an NMR imaging-effective amount of a complex according to any one of claims 7 to 9.

11. A composition according to claim 10, which is suitable for parenteral administration, wherein the complex is dissolved or suspended in a sterile aqueous pharmaceutically acceptable carrier at a concentration of from about 0.05 to 1.0M.

12. A composition according to claim 10 or claim 11, which further contains a pharmaceutically acceptable buffer.

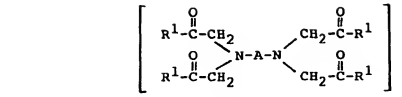
13. A composition according to any one of claims 10 to 12, which further contains a pharmaceutically acceptable electrolyte.

14. A composition according to any one of claims 10 to 13, which further comprises a salt of a complexing agent according to any one of claims 1 to 6, the cation(s) of said salt being one or more physiologically acceptable, non-toxic cations.

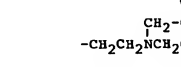
15. A composition according to claim 14, wherein said complexing agent is employed in an amount ranging from about 0.1 to about 15 mole % of the paramagnetic ion containing complex, and is complexed with one or more cations selected from the group consisting of sodium ions, calcium ions, magnesium ions, zinc ions and mixtures thereof.

Patentansprüche

1. Komplexbildner der Formel:



wobei A aus der Gruppe bestehend aus $-CH_2CH_2-$ und



ausgewählt wird, wobei die R^1-Gruppen gleich oder verschieden sein können und aus der Gruppe bestehend aus -OH und

ausgewählt werden, wobei R^2 ein Alkoxyalkyl ist, bei dem der Alkoxyanteil 1 oder 2 Kohlenstoffatome enthält und der Alkylanteil 2 bis 5 Kohlenstoffatome enthält, oder $-(CH_2CH_2O)_n-R_4$ ist, wobei n 1-10 ist und R_4 H (außer wenn n = 1), ein Alkyl mit 1 bis 8 Kohlenstoffatomen oder ein nichtsubstituiertes oder mit Hydroxy substituiertes Aryl ist, und R^3 H, R^2, ein Alkyl mit 1 bis 8 Kohlenstoffatomen, Hydroxy, ein Alkoxy mit 1 bis 8 Kohlenstoffatomen, ein Cycloalkyl mit bis zu 10 Kohlenstoffatomen oder eine Arylgruppe ist, die wahlweise mit Hydroxy, Carboxyl, Halogen, einem Alkoxy mit 1 bis 8 Kohlenstoffatomen oder einem Alkyl mit 1 bis 8 Kohlenstoffatomen substituiert ist, wobei 2 oder 3 der R^1-Gruppen -OH sind und der Rest der R^1-Gruppen

ist.

2. Komplexbildner nach Anspruch 1, wobei R^1 Methoxymethylamino, Methoxypropylamino, Methoxybutylamino, Methoxypentylamino, Ethoxyethylamino, Ethoxypropylamino oder Ethoxybutylamino ist.

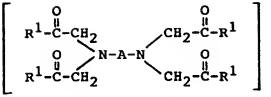
3. Komplexbildner nach Anspruch 1, wobei R^2 $-(CH_2CH_2O)_n-R^4$ ist, n 1, 2 oder 3 ist und R^4 H (außer wenn n = 1) oder eine Alkylgruppe mit 1 bis 5 Kohlenstoffatomen ist.

4. Komplexbildner nach einem der Ansprüche 1 bis 3, wobei R^3 H ist.

5. Komplexbildner nach einem der Ansprüche 1 bis 3, wobei R^3 R^2, ein Alkyl mit 1 bis 8 Kohlenstoffatomen, Hydroxy oder eine Alkoxygruppe mit 1 bis 8 Kohlenstoffatomen ist.

6. Komplexbildner nach einem der Ansprüche 1 bis 3, wobei R^3 eine Arylgruppe ist, die wahlweise mit Hydroxy, Carboxyl, Halogen, einem Alkoxy mit 1 bis 8 Kohlenstoffatomen oder einem Alkyl mit 1 bis 8 Kohlenstoffatomen substituiert ist.

7. Komplex aus einem Komplexbildner nach einem der Ansprüche 1 bis 6 und einem Metallion M^{z+} mit der folgenden Formel:



M^{+z}

wobei A die ihm in Anspruch 1 zugeschriebene Bedeutung hat und R^1

wie in Anspruch 1 definiert oder -O⁻ ist und M^{+z} ein paramagnetisches Ion eines Elements mit der Ordnungszahl 21-29, 42-44 oder 58-70 und einer Valenz Z von +2 oder +3 ist.

8. Komplex nach Anspruch 7, wobei M^{+z} Chrom(III), Mangan(II), Mangan(III), Eisen(III), Eisen(II), Cobalt(II), Nickel (II), Kupfer(II), Praseodym(III), Neodym(III), Samarium(III), Ytterbium(III), Gadolinium(III), Terbium(III), Dysprosium (III), Holmium(III) oder Erbium(III) ist.

9. Komplex nach Anspruch 7 oder Anspruch 8, wobei R^1 Methoxyethylamino ist und M^{+z} Gadolinium(III) ist.

10. Diagnostische Zusammensetzung, geeignet zur enteralen oder parenteralen Verabreichung an ein warmblütiges Tier, welche eine NMR-tomographisch wirksame Menge eines Komplexes nach einem der Ansprüche 7 bis 9 umfaßt.

11. Zusammensetzung nach Anspruch 10, die geeignet ist zur parenteralen Verabreichung, wobei der Komplex in einem sterilen wässerigen pharmazeutisch annehmbaren Träger mit einer Konzentration von etwa 0,05 bis 1,0 M gelöst oder suspendiert ist.

12. Zusammensetzung nach Anspruch 10 oder Anspruch 11, die außerdem einen pharmazeutisch annehmbaren Puffer enthält.

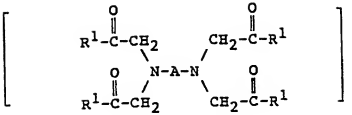
13. Zusammensetzung nach einem der Ansprüche 10 bis 12, die außerdem einen pharmazeutisch annehmbaren Elektrolyten enthält.

14. Zusammensetzung nach einem der Ansprüche 10 bis 13, die außerdem ein Salz eines Komplexbildners nach einem der Ansprüche 1 bis 6 enthält, wobei das/die Kation(en) dieses Salzes ein oder mehrere physiologisch annehmbare, nicht toxische Kationen sind.

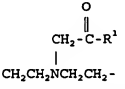
15. Zusammensetzung nach Anspruch 14, wobei der Komplexbildner in einer Menge von etwa 0,1 bis etwa 15 Mol-% des das paramagnetische Ion enthaltenden Komplexes verwendet wird und mit einem oder mehreren Kationen, ausgewählt aus der Gruppe bestehend aus Natriumionen, Calciumionen, Magnesiumionen, Zinkionen oder Mischungen davon, komplexiert ist.

Revendications

1. Agent complexant de formule:



dans laquelle A est choisi dans le groupe constitué par $-CH_2CH_2-$ et



dans laquelle les groupes R^1 peuvent être identiques ou différents et sont choisis dans le groupe constitué par -OH et

dans laquelle R^2 est un alcoxyalkyle dans lequel la partie alcoxy contient 1 ou 2 atomes de carbone et la partie alkyle contient de 2 à 5 atomes de carbone, ou est $-(CH_2CH_2O)_n-R^4$ dans laquelle n est 1-10 et R^4 est H (sauf lorsque n = 1), un alkyle ayant de 1 à 8 atomes de carbone, ou un aryle non substitué ou substitué avec un hydroxy et R^3 est H, R^2, un alkyle ayant de 1 à 8 atomes de carbone, un hydroxy, un alcoxy ayant de 1 à 8 atomes de carbone, un cycloalkyle ayant jusqu'à 10 atomes de carbone, ou un groupe aryle qui est facultativement substitué avec un hydroxy, un carboxyle, un halogène, un alcoxy ayant de 1 à 8 atomes de carbone ou un alkyle ayant de 1 à 8 atomes de carbone, dans laquelle 2 ou 3 des groupes R^1 sont -OH et les groupes R^1 restants sont

2. Agent complexant selon la revendication 1, dans lequel R^1 est le méthoxyéthylamino, le méthoxypropylamino, le méthoxybutylamino, le méthoxypentylamino, l'éthoxyéthylamino, l'éthoxypropylamino ou l'éthoxybutylamino.

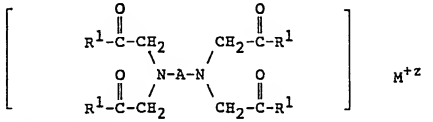
3. Agent complexant selon la revendication 1, dans lequel R^2 est $-(CH_2CH_2O)_n-R^4$, n est 1, 2 ou 3 et R^4 est H (sauf lorsque n = 1) ou un groupe alkyle ayant de 1 à 5 atomes de carbone.

4. Agent complexant selon l'une quelconque des revendications 1 à 3, dans lequel R^3 est H.

5. Agent complexant selon l'une quelconque des revendications 1 à 3, dans lequel R^3 est R^2, un alkyle ayant de 1 à 8 atomes de carbone, un hydroxy, ou un groupe alcoxy ayant de 1 à 8 atomes de carbone.

6. Agent complexant selon l'une quelconque des revendications 1 à 3, dans lequel R^3 est un groupe aryle facultativement substitué avec un hydroxy, un carboxyle, un halogène, un alcoxy ayant de 1 à 8 atomes de carbone ou un alkyle ayant de 1 à 8 atomes de carbone.

7. Complexe d'un agent complexant selon l'une quelconque des revendications 1 à 6 et d'un ion métallique M^{z+} et ayant la formule suivante:



dans laquelle A possède la signification qui lui est attribuée dans la revendication 1 et R^1 est

comme défini dans la revendication 1 ou est -O⁻, et M^{+z} est un ion paramagnétique d'un élément ayant un numéro atomique de 21-29, 42-44 ou 58-70, et une valence, Z, de +2 ou +3.

8. Complexe selon la revendication 7, dans lequel M^{+z} est le chrome (III), le manganèse (II), le manganèse (III), le fer (III), le fer (II), le cobalt (II), le nickel (II), le cuivre (II), le praséodyme (III), le néodyme (III), le samarium (III), l'ytterbium (III), le gadolinium (III), le terbium (III), le dysprosium (III), l'holmium (III) ou l'erbium (III).

9. Complexe selon la revendication 7 ou la revendication 8, dans lequel R^1 est le méthoxyéthylamino et M^{+z} est le gadolinium (III).

10. Composition de diagnostic convenant à une administration entérale ou parentérale chez un animal à sang chaud, qui comprend une quantité efficace pour l'imagerie RMN d'un complexe selon l'une quelconque des revendications 7 à 9.

11. Composition selon la revendication 10, convenant à une administration parentérale, dans laquelle le complexe est dissous ou mis en suspension dans un support aqueux stérile pharmaceutiquement acceptable à une concentration d'environ 0,05 à 1,0M.

12. Composition selon la revendication 10 ou la revendication 11, contenant, en outre, un tampon pharmaceutiquement acceptable.

13. Composition selon l'une quelconque des revendications 10 à 12, contenant, en outre, un électrolyte pharmaceutiquement acceptable.

14. Composition selon l'une quelconque des revendications 10 à 13, contenant, en outre, un sel d'un agent complexant selon l'une quelconque des revendications 1 à 6, le(s) cation(s) dudit sel étant un (des) cation(s) physiologiquement acceptable(s), non toxique(s).

15. Composition selon la revendication 14, dans laquelle ledit agent complexant est utilisé en une quantité allant d'environ 0,1 à environ 15 moles % du complexe contenant l'ion paramagnétique, et est complexé avec un ou plusieurs cations choisis dans le groupe constitué par des ions sodium, des ions calcium, des ions magnésium, des ions zinc et leurs mélanges.